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A Study of the Oxygen Consumption of Blind and Eyed Cave Characins in Light and in Darkness

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(Text-figures 1 & 2.)

INTRODUCTION.

Certain features of the Mexican cave characins and their surface dwelling relatives make them particularly useful in studies of comparative behavior concerning the effects of vision or its lack. The normally blind cave form described as *Anoptichthys jordani* by Hubbs and Innes (1936) is genetically continuous with the deriving form, *Astyanax mexicanus* (Filippi), as has been indicated by Breder (1942). Laboratory studies by Breder and Gresser (1941a, 1941b) and Breder and Rasquin (1943) have shown that the locomotor activity of the eyed and blind fishes is distinctly different. Those with vision remain for the most part in a quiescent aggregation, while both the normally and operationally blind individuals maintain a continuous random wandering. The important role of vision in the schooling behavior of all fish species so far studied, is well known, as is the fact that the other senses provide for only a limited aggregating tendency.

The above-mentioned studies apply to fishes in the presence of light since it is manifestly impossible to make direct observations in total darkness. Since Breder and Gresser (1941a) have shown that the blind cave fish are negatively phototropic and Breder and Rasquin (1943) that blinded river fish take on the wandering characteristic of the normally blind cave fish, it becomes obviously desirable to obtain some estimate of what such fishes actually do in a locomotor sense in an environment of total darkness. It is the purpose of this study to determine whether or not there is a measurable differential in the activity of both blind and eyed fishes in light and in darkness. Since there is, in general, a proportionality between oxygen consumption and activity of a fairly sensitive degree, as has been indicated by Schlaifer (1938), a method comparable to his has been employed.

In addition to the above main purpose of the study it has also been possible to compare the behavior of such fishes alone and in groups. Some fishes have differing oxygen consumption rates, dependent on their degree of isolation, as has been indicated by Schlaifer (1939). Furthermore, he was able to determine that goldfish have a distinctly higher rate of oxygen consumption in the light than in the dark. Shaw, Escobar and Baldwin (1938) showed that the locomotor activity of the goldfish is reduced over a period of 96 hours to one-half or one-third the usual rate by very low illumination.

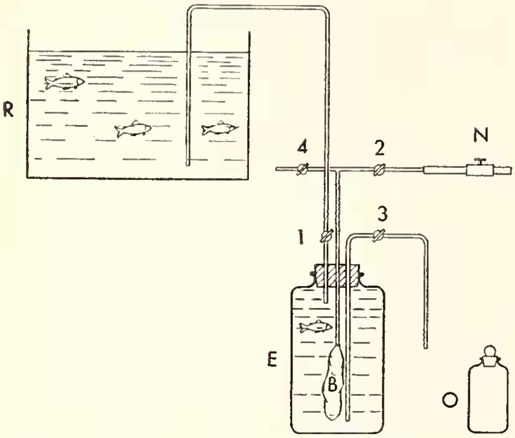
The basic question concerned with whether there is a fundamental difference in the metabolic rate of the blind and eyed fish, or whether any detectable differences are merely referable to the presence or absence of vision, is covered by experiments involved in work on the preceding items. The results and their significance in terms of differences between these types of fishes, their comparison with goldfish and the problem of cave entry and establishment therein in ecological and evolutionary terms, is covered in the discussion.

The experimental part of this study was carried out in the laboratories of the Department of Animal Behavior of the American Museum of Natural History through the courtesy of the department's Chairman, Dr. Frank A. Beach.

MATERIALS AND METHODS.

Determinations of dissolved oxygen were made by means of the permanganate modification of the Winkler method. This modified technique, suggested by Allee and Oesting (1934) and described in the American Public Health Association Manual (1943), eliminates the error caused by nitrites in the water.

The apparatus devised for these tests is shown in Text-fig. 1, the structural details of which may be briefly summarized as follows. In use the test chamber E is filled with water from reservoir R. The animal undergoing test is introduced into chamber E and allowed to remain there for a minimum of 17 hours. This period enables the fish to acclimate itself to the experimental surroundings. Chamber E, which is a two liter jar, is then filled to overflowing and a rubber stopper, through which pass three glass tubes, is inserted, making certain that no air bubbles are trapped. Needle valve N is set to admit air very slowly. With stop cocks 4 and 3 closed, 1 and 2 are opened. This causes balloon B to expand and drive water back into reservoir R. If an air bubble is accidently caught in the glass tubing leading from the reservoir to the animal chamber, it is thus voided into the open reservoir. With cocks 2 and 3 closed, 4 and 1 are opened. This causes the balloon to deflate and water returns to E with no entrained air. With cocks 2 and 4 closed, 1 and 3 are opened. This causes a flow from R through E and out of the outlet tube. This flow is allowed to continue until E has cleared itself thoroughly.



TEXT-FIG. 1. Diagram of apparatus used to determine oxygen consumption of blind and eyed characins. **B** = balloon, **E** = experimental chamber, **N** = needle valve, **O** = water sample bottle, **R** = reservoir of water supply, **1**, **2**, **3**, **4** = glass stop cocks.

Samples of water for analysis are collected in 250 cc. bottles as suggested by American Public Health Association Manual (1943). To withdraw a sample the outlet tube is allowed to reach down to the bottom of bottle O. The first sample is collected after E is thoroughly cleared, by merely allowing the flow to continue from R through E and out of the outlet tube into the sample bottle. The water is allowed to overflow in the sample bottle in order to eliminate the surface water which is contaminated by the air that is present in the bottle before filling. All the stop cocks are then closed and the apparatus is in operating condition. To collect a sample at the end of the test period stop cocks 3 and 2 are opened so that the balloon expands slowly and water passes out of the outlet into the sample bottle. Before the sample is actually collected, however, a small amount of water is allowed to run to waste equal to that in the outlet pipe. To reset the apparatus stop cocks 2 and 3 are closed whereas 1 and 4 are opened. When the balloon is deflated cock 4 is closed and 3 is opened in order to flush out the old contents of animal chamber E. The difference between the oxygen content of the water in the animal chamber at the start of the test and at the completion of the test is a measure of the oxygen consumption of the fish. The water in the reservoir was kept in a conditioned state throughout the experiment by using it as a storage space for reserve fish.

When the experiments were performed involving total darkness, test chamber E was placed in a tall can which had been painted black on the inside as well as the outside. A covering, made of several layers of heavy black cloth, was fitted over the can and designed to snugly enclose the top of chamber E. The stop cocks and glass tubing leading directly to and from the test chamber were completely covered with black tape. When the apparatus was in operating condition, a large piece of heavy black cloth was thrown over the entire apparatus. Thus light was effectively excluded without sacrificing any of the necessary controls.

This closed system type of respirometer was employed in spite of the objection that the closed method allows the products of metabolism to accumulate and the CO_2 tension to increase, thus modifying the oxygen consumption readings. The continuous flow respirometers used by Keys (1930), Etkin, Root and Mofshin (1940), Wells (1935), Adkins (1930), and others eliminate these uncertainties but similarly are not absolute measures of the oxygen consumption of the animal. In all of these latter methods the rate of flow varies according to the operator and the oxygen consumption reading is directly dependent upon how fast or how slow the water is allowed to circulate through the system. Thus the oxygen consumption reading is always a relative figure. Since both methods apparently possess their own peculiar disadvantages and advantages, it was decided to employ the much simpler closed system type of respirometer for the present work. The fact that the values obtained are not absolute is hardly important because the problem at hand involves only a comparison of oxygen consumption rates. Any modification in readings should be consistent throughout.

Schlaifer (1938) also employed the closed system type of respirometer in determining the oxygen consumption of goldfish. Heavy mineral oil was used by him to prevent the leakage of oxygen from the air into the water during the experiments. The main disadvantage of this method, as discussed by Schlaifer, is that mineral oil is not a perfect seal. He showed that there was a leakage of 1.15 per cent. to 1.55 per cent. of the total oxygen available in both his 6 liter and

12 liter tanks in three hours. However, since he was interested in comparative values, his conclusions were not altered. In the present work it was decided not to use this method, in spite of the fact that comparative data were also sought, since mineral oil might prove very costly to the test fish. If the animal swam to the interface between the oil and water, the gills could become clogged with oil and death might result.

The balloon, which is used in the present work to force the water into and out of the test chamber, seems to eliminate most of the disadvantages of previously used systems and in addition is much cleaner and more easily operated, the entire control being governed by five valves.

The fishes chosen for test were selected so as to be comparable in size and condition. Their length was between 4.2 cm. and 6.0 cm. in standard length, and their weight was between 2.7 g. and 5.6 g. Each fish was introduced into the test chamber at least 17 hours before respiration readings were begun. Each fish was starved for 17 hours before the test. The temperature throughout this work ranged from 22° to 29.5° C.; however, the great majority of the readings were taken at 27° C. Since the hydrogen ion concentration may affect the metabolism of fishes (Wells, 1935), the pH of the test water was maintained at 7 in all but a very few cases where it was 6.9.

Tests were planned as listed below on optically intact river fish.

1. One fish tested in daylight.
2. One fish tested in total darkness.
3. Two fish in daylight.
4. Two fish in total darkness.
5. Five immature optically intact fish, total weight 6.1 g., in daylight.
6. Five immature optically intact fish in total darkness.

A similar series of experiments was carried on with blind cave fish. The length of the test period in each case was two hours. This time limit was decided upon after carrying on a series of preliminary experiments on eyed and on blind specimens, for it was found that one hour readings obtained on the eyed river fish were too small to be entirely satisfactory but the two hour readings appeared to be adequate.

A blank test was run with each experiment in order to determine the biochemical demand of the conditioned water. Each experiment was repeated ten times and the values as finally used represent the means of these ten tests. In all 155 tests were made, representing a total of 301 hours measured as to oxygen consumption. In all nine eyed fish and eight blind fish were employed.

EXPERIMENTAL RESULTS.

While working with the eyed river fish two very distinct and sharply defined phases of behavior were noted; a turbulent fighting state and a quiescent state. The probable explanation of this is given in the discussion. Oxygen consumption readings were obtained on a single fish (No. 1) in both the quiet and the excited states. This change, over a period of five days, is reflected in Table I. This fish continued to be restless and in a highly excited state, giving high oxygen consumption readings during the remainder of this work.

Effect of darkness on oxygen consumption of eyed fish.

Ten additional tests were performed on eyed fish No. 1 in the light and ten tests in total darkness. This fish was in an active state throughout this series of tests. It was the purpose of these experiments to discover the extent of activity in the dark. Evidently three possibilities are present. Darkness could cause the active eyed fish to return to the resting state. This would be indicated by a lowered oxygen consumption similar to the decrease obtained with goldfish by Schlaifer (1939). One other possibility is that the eyed fish, once unable to see, would enter into the continuous wandering state characteristic of their blind relatives as found in blinded specimens by Breder and Rasquin (1943), or there finally could be the third alternative of no change whatsoever. The results of 20 oxygen consumption tests are shown in Table II, which indicates that less oxygen was consumed in the dark.

TABLE I. EYED FISH NO. 1.

Transition from quiet to active state over a period of five days.

S. L. = 5.8 cm. WT. = 4.4 g.

Tested in daylight.

Date	Temp.	Test Period Hours	cc. O_2/1 Original Sample	cc. O_2/1 Consumed
7/30	27°	2	3.35	.49
7/30	27°	1	3.14	.14
7/30	27°	1	4.19	.28
7/31	26°	2	4.95	.76
7/31	26°	2	4.95	.62
7/31	26°	1	5.09	.41
8/1	25.5°	2	4.22	1.29
8/1	25.5°	2	4.05	1.19
8/1	25.5°	1	4.43	.84
8/1	26°	1	4.36	.52
8/2	25°	2	4.61	1.19
8/2	25.5°	2	4.22	1.43
8/2	26°	2	3.91	1.43
8/3	26°	2	3.63	1.40
8/3	26°	2	3.49	1.18
8/3	26°	2	3.42	1.33

Schlaifer (1938) showed that two goldfish living together exhibit what has been called the "group effect." This is a condition in which animal aggregations either in nature or in the confines of experimental apparatus show different physiological responses as compared with isolated animals of the same species. Escobar, Minahan and Shaw (1936), in their discussion of "group effect," point out that this phenomenon has been shown for differential growth rates, reproductive rates, conditioned learning times, locomotor activity, oxygen consumption rates, etc. Schuett (1933) showed that the oxygen consumption of a goldfish is higher when isolated in a given volume of water than when it is a member of a group of four in the same volume of water. Schuett (1934) corrected an error in his water analysis technique of 1933. Nevertheless conclusions similar to Schuett's 1933 work were drawn by Schlaifer (1938). In Schlaifer's experiments two goldfish living together consumed less oxygen per fish than when isolated. Escobar, Minahan and Shaw (1936) point out that the lowered oxygen consumption of a grouped fish is probably due to the fact that the activity of an isolated animal is greater than the activity of the same animal when grouped with other fish. Breder and Nigrelli (1938) confirmed this latter fact, using larger bodies of water.

TABLE II. EYED FISH NO. 1 IN AN ACTIVE STATE.

S. L. = 5.8 cm. WT. = 4.4 g.

All tests run for 2 hours. pH 7 in all cases.

A. Tested in daylight.

Date	Temp.	cc. 0_2/1 Original Sample	cc. 0_2/1 Consumed in 2 Hours
8/8	25.5°	4.40	1.19
8/8	26°	4.12	.98
8/8	26°	4.19	.98
8/9	25.5°	3.70	1.03
8/9	26°	3.63	1.26
8/9	26°	3.70	1.12
8/10	26°	4.12	.98
8/10	27°	3.91	1.54
8/10	27°	4.19	.98
8/11	27°	4.19	1.40
Average		4.02	1.15

Average B.O.D. = + .03 cc. 0_2/1

B. Tested in total darkness.

Date	Temp.	cc. 0_2/1 Original Sample	cc. 0_2/1 Consumed in 2 Hours
8/25	22.5°	4.95	.83
8/25	22°	5.02	.55
8/27	22.5°	4.95	1.46
8/27	23°	4.75	.84
8/27	23°	4.89	.63
8/28	23°	4.89	.70
8/28	24°	4.89	.56
8/28	24°	4.95	.76
8/29	25°	4.19	1.40
8/29	26°	4.26	1.47
Average		4.77	0.92

Average B.O.D. = + .03 cc. 0_2/1

An attempt was made to measure the oxygen consumption of two optically intact fish in order to determine whether they too exhibit the group effect. The animals chosen for test were those which were removed from the reserve tank and placed in individual tanks ten days previous because they were in an active fighting state. Throughout the ten-day isolation period they remained quiet in their respective tanks, resembling those in the quiescent state. They were placed in the respirometer for an acclimatization period. Although they were both of approximately the same size, one fish attacked the other and succeeded in killing it. A new pair of fish, which had been living in the same tank, undisturbed since hatching, was then placed in the respirometer. During the acclimatization period they too exhibited fighting qualities, pecking at and chasing each other. Since this was evidently leading to destruction, they were separated. Apparently the respirometer, which is a 2 liter chamber, brings the fish into too close confinement for them to establish normal social relationships. It was thus impossible to test two optically intact river fish in the light by means of this device. However, when two optically intact fish, which fought in the light, were placed in the same respirometer in total darkness the destructive activity was completely eliminated. They survived the four-day test period and at this writing are still living. When unable to see, they are likewise unable to destroy each other. These consumed a mean of 0.87 cc. 0_2 per liter per fish. The results of ten respiratory metabolism tests on two optically intact river fish in complete darkness are shown in Table III.

A single optically intact fish, No. 4, which

TABLE III. TWO EYED FISH, NO. 2 AND NO. 3, IN AN ACTIVE STATE TESTED IN TOTAL DARKNESS.

No. 2: S. L. = 5.6 cm. WT. = 4 g.

No. 3: S. L. = 6.0 cm. WT. = 5.6 g.

All tests run for 2 hours. pH 7 in all but last which was 6.9.

Date	Temp.	cc. 0_2/1 Original Sample	cc. 0_2/1 Consumed
8/27	23°	5.16	1.25
8/28	23°	4.89	1.61
8/28	24°	4.54	1.54
8/28	24°	4.75	1.19
8/29	25°	4.54	1.96
8/29	26°	4.47	1.68
8/29	27°	4.40	1.54
8/29	27°	4.72	1.91
8/30	27°	3.84	2.10
8/30	27°	4.26	2.52
Average		4.56	1.73 = .87 per fish

Average B.O.D. = + .06 cc. 0_2/1

had been living alone since hatching, was tested in the respirometer. This fish remained in the normal quiescent state throughout the test period. It consumed a mean of 0.63 cc. O_2 per liter in the light and 0.73 cc. O_2 per liter in darkness, thus showing an increase in consumption. The results of ten tests in the light and ten tests in the dark are shown in Table IV.

Ten tests were performed on five immature optically intact fish in the light and ten tests in total darkness. It was possible to make such tests in the light because the fighting previously mentioned does not appear before maturity is reached. They consumed a mean of 0.22 cc. O_2 per liter per fish in the light and 0.28 cc. O_2 per liter per fish in darkness.

Effect of darkness on oxygen consumption of blind fish.

The respiratory metabolism of a single blind fish was taken in the daylight and in total darkness. This fish consumed a mean of 1.30 cc. O_2 per liter in the light and 1.02 cc. O_2 per liter in darkness. The results of ten tests in the light and ten tests in the dark are shown in Table VI.

Two blind fish were tested in the respirometer in daylight and in total darkness. Unlike the paired eyed fish they were not at all disturbed in the 2 liter test jar. They consumed a mean of 0.98 cc. O_2 per liter per fish in the light and 0.93 cc. O_2 per liter per fish in darkness. The results are shown in Table VII.

TABLE IV. EYED FISH NO. 4 IN A PASSIVE STATE.

S. L. = 5.5 cm. WT. = 3.8 g.

All tests run for 2 hours. pH 7 in all cases.

A. Tested in daylight.

Date	Temp.	cc. O_2/1 Original Sample	cc. O_2/1 Consumed
8/21	27°	3.14	.63
8/21	27°	3.42	.77
8/22	27°	3.70	.77
8/22	27°	3.35	.63
8/22	27°	3.98	.70
8/23	26.5°	4.19	.49
8/23	26.5°	4.95	.60
8/23	26.5°	4.75	.56
8/24	23°	4.75	.70
8/24	23°	5.23	.41
Average		4.15	.63

Average B.O.D. = + .03 cc. O_2/1

B. Tested in total darkness.

Date	Temp.	cc. O_2/1 Original Sample	cc. O_2/1 Consumed
8/25	22°	5.09	1.04
8/25	22.5°	5.02	.69
8/25	22°	5.02	.54
8/27	22.5°	5.16	.90
8/27	23°	4.89	.56
8/27	23°	5.09	.62
8/28	23°	4.95	.76
8/28	24°	5.16	.62
8/28	24°	4.89	.84
8/29	25°	4.47	.77
Average		4.97	.73

Average B.O.D. = + .03 cc. O_2/1

TABLE V. TESTS ON FIVE IMMATURE OPTICALLY INTACT FISH.

Average S. L. = 3.0 cm.

Total WT. = 6.1 g.

All tests run for 2 hours. pH 7 in all cases.

A. Tested in daylight.

Date	Temp.	cc. O_2/1 Original Sample	cc. O_2/1 Consumed
8/23	26.5°	4.26	.98
8/23	26.5°	4.68	1.19
8/23	26.5°	4.61	.91
8/24	23°	4.54	1.05
8/24	23°	5.16	1.46
8/24	23°	5.51	.90
8/25	22°	5.16	1.11
8/25	22.5°	4.95	.83
8/25	22°	5.23	1.25
8/27	22.5°	5.02	1.18
Average		4.91	1.09

Average B.O.D. = + .03 cc. O_2/1

B. Tested in total darkness.

Date	Temp.	cc. O_2/1 Original Sample	cc. O_2/1 Consumed
8/29	27°	4.72	1.02
8/29	27°	4.95	.97
8/30	27°	4.33	1.26
8/30	27°	4.40	1.33
8/30	28°	4.75	1.26
8/30	29°	4.47	1.47
8/31	28°	4.61	1.75
8/31	29.5°	4.19	1.33
8/31	29.5°	3.91	1.40
9/ 1	28°	4.19	1.96
Average		4.45	1.36

Average B.O.D. = + .03 cc. O_2/1

Five immature blind fish, approximately the same size as the five immature eyed fish, were tested in daylight and in complete darkness. They consumed a mean of 0.27 cc. O_2 per liter per fish in the light and 0.24 cc. O_2 per liter per fish in darkness. The results are shown in Table VIII.

The minimum, maximum and mean values of Tables II to VIII are summarized in Table IX. A graphic presentation of the minimum, mean and maximum values is made in Text-fig. 2. This shows more clearly the relationships involved.

DISCUSSION.

During the early experiments the eyed river fish rested quietly in schools, showing no signs of movement other than those necessary to carry on the respiratory process. This quiescent state is normal to this species and is described in detail by Breder and Gresser (1941a). After several days of experimentation the reserve fish in the reservoir tank suddenly lost this quiet aggregating tendency. They chased each other about the tank and picked at one another. Their entire social attitude was altered. A few of these fish soon died. On examination it was found that the caudal fin was injured in a few cases and in others the gills were frayed. The remaining fish, which continued chasing one another, were separated into individual tanks to prevent further destruction. Nothing had been added to the tank and nothing had been changed. These reserve fish had been fed routinely each day. This same phenomenon was noted by Breder (1943) who wrote: "This school of fish [*Astyanax mexicanus* (Filippi)] grew and fed voraciously, showing full vigor until November 1. On routine examination of the aquaria it was found on the morning of the next day that most of the fish were dead and about half a dozen, still alive, were huddled in a tight school and very evidently in serious difficulty, showing their darkest phase. Later that day they too expired. On examination, each fish was seen to have its tail fin at least half gone. There was nothing else present in the aquarium except sand, aquatic plants and an aeration tube. Six other aquaria containing sibs of these, in greater or less numbers, were all in perfect order as all of them had been for months.

"Whatever happened in this aquarium is not entirely clear but it evidently triggered off something in the nature of a 'free for all' in which not a single fish triumphed or survived. There was no question of lack of food as they had been fed each day, including the morning of November 1. Although I have had a considerable experience with a wide variety of fishes in captivity no such sudden or complete self destruction had been previously experienced."

TABLE VI. BLIND FISH NO. 5.

S. L. = 5.3 cm. WT. = 3 g.

All tests run for 2 hours. pH 7 in all cases.

A. Tested in daylight.

Date	Temp.	cc. 0_2/1 Original Sample	cc. 0_2/1 Consumed
8/25	22°	5.16	1.18
8/25	22.5°	5.02	1.67
8/25	22°	4.95	1.11
8/27	22.5°	5.02	.90
8/27	23°	5.02	1.25
8/27	23°	4.89	1.40
8/28	23°	4.69	1.54
8/28	24°	4.89	1.40
8/28	24°	4.68	1.12
8/29	25°	4.54	1.40
Average		4.89	1.30

Average B.O.D. = + .03 cc. 0_2/1

B. Tested in total darkness.

Date	Temp.	cc. 0_2/1 Original Sample	cc. 0_2/1 Consumed
8/21	27°	3.49	.56
8/21	27°	3.33	.70
8/22	27°	3.63	.77
8/22	27°	3.35	1.12
8/22	27°	3.77	1.26
8/23	26.5°	4.19	1.40
8/23	26.5°	4.33	.63
8/23	26.5°	4.95	1.11
8/24	23°	4.68	1.26
8/24	23°	5.37	1.40
Average		4.11	1.02

Average B.O.D. = + .03 cc. 0_2/1

TABLE VII. TWO BLIND FISH, NO. 6, AND NO. 7

No. 6: S. L. = 4.9 cm. WT. = 2.7 g.

No. 7: S. L. = 6.0 cm. WT. = 4.2 g.

All tests run for 2 hours. pH 7 in all cases.

A. Tested in daylight.

Date	Temp.	cc. 0_2/1 Original Sample	cc. 0_2/1 Consumed
8/29	26°	4.47	1.61
8/29	27°	4.82	2.20
8/29	27°	4.61	1.12
8/30	27°	4.40	1.82
8/30	27°	4.26	1.75
8/30	28°	4.40	2.24
8/30	29°	4.26	2.24
8/31	28°	4.40	2.31
8/31	29.5°	3.70	2.24
8/31	29.5°	3.49	1.96
Average		4.28	1.95

Average B.O.D. = – .01 cc. 0_2/1

B. Tested in total darkness.

Date	Temp.	cc. 0_2/1 Original Sample	cc. 0_2/1 Consumed
8/22	27°	3.49	2.30
8/22	27°	2.79	1.88
8/22	27°	3.63	2.72
8/23	26.5°	3.97	2.09
8/23	26.5°	4.68	1.61
8/23	26.5°	4.89	1.26
8/24	23°	4.68	2.03
8/24	23°	4.89	1.47
8/24	23°	5.51	1.88
8/24	23°	5.02	1.32
Average		4.36	1.86

Average B.O.D. = + .02 cc. 0_2/1

Since this same peculiarity was noted on two different occasions with no apparent reason, it may mean that this sudden appearance of fighting is inherent in the life cycle of the species under certain conditions or that they are so sensitive to slight disturbances that, as in the present case, transference to the reservoir tank from the tank in which they had been living since hatching initiated this change.

The mean daily readings of oxygen consumption of the specimen which was measured during its change from a passive to an active state, suitably adjusted, are given graphically in Text-fig. 2. It will be noted that the comparative means given for other fish show that this specimen passed, between the second and third day, from the range of eyed fish in the resting stage to the range of

TABLE VIII. FIVE IMMATURE BLIND FISH.

Average S. L. = 3.0 cm.
Total WT. = 2.1 g.
All tests run for 2 hours. pH 7 in all cases.

A. Tested in daylight.

Date	Temp.	cc. O_2/l Original Sample	cc. O_2/l Consumed
8/17	27°	3.77	1.12
8/17	27°	3.70	1.40
8/20	26.5°	3.84	.84
8/20	27°	3.56	.77
8/20	27°	3.98	1.05
8/21	27°	3.49	2.23
8/21	27°	3.35	2.02
8/21	27°	3.21	1.68
8/22	27°	3.63	1.19
8/22	27°	3.35	1.33
Average		3.59	1.36

Average B.O.D. = + .08 cc. O_2/l

B. Tested in total darkness.

8/29	26°	4.72	1.23
8/29	27°	4.89	.98
8/29	27°	4.89	.70
8/30	27°	4.19	.91
8/30	27°	4.61	1.12
8/30	28°	4.82	1.19
8/30	29°	4.61	1.26
8/31	28°	4.61	1.40
8/31	29.5°	4.33	1.54
8/31	29.5°	4.19	1.47
Average		4.59	1.18

Average B.O.D. = – .01 cc. O_2/l

not only other active eyed fish but also to that normal to the blind fish.

The data of Tables II and III indicate that darkness has a quieting effect on the eyed fish when they are in the highly active, turbulent state, similar to that on the blind fish. However, the oxygen consumption readings in the dark do not reach the low values characteristic of these fish in the normal resting state as is shown in Text-fig. 2.

Eyed fish No. 4, which remained in the "normal" quiescent state throughout the experiments, on the other hand, consumed more oxygen in the dark, as shown in Table IV, than in the light. This indicates that the eyed fish is swimming more actively in the dark in the absence of any possible visual fixation. Apparently the eyed fish, when unable to see, resort to a wandering motion characteristic of the blind individuals. Breder and Rasquin (1943) showed that eyed river fish in which the optic nerve had been severed took on the essential wandering behavior of the naturally blind fish. One may conclude from this that the contrasting locomotor behavior in the genetically related blind and eyed fish is a matter of vision and its lack. Schlaifer (1939) found that an isolated goldfish shows a higher rate of oxygen consumption in the light than in the dark. The isolated animal, subjected to no stimuli from other fishes, is affected only by the change in illumination, and its activity in the dark is diminished. The effect of such a change in illumination on the eyed characins, under similar conditions, is exactly opposite. Evidently this difference is rooted in the inherent difference between the behavior of the two species, the characins normally remaining quiet and the goldfish generally moving about.

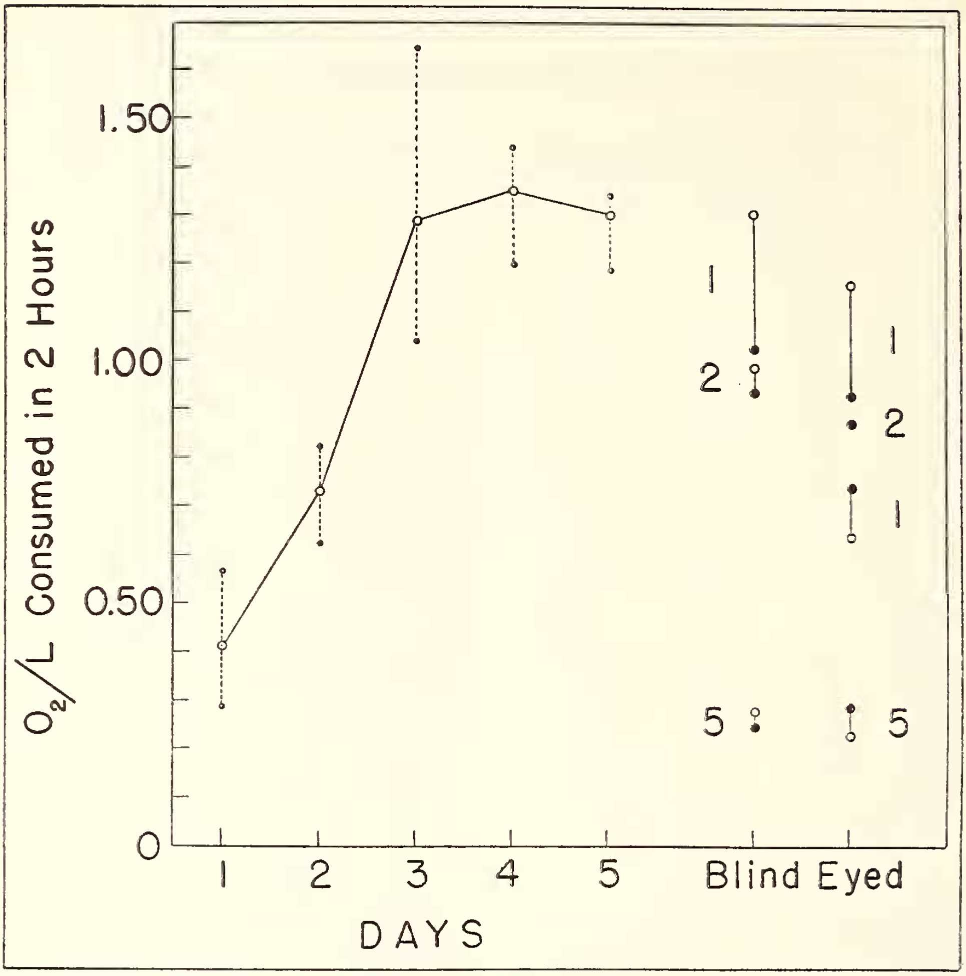
Table V shows that five immature optically intact fish consume more oxygen in the dark than in the light. This agrees with the results obtained in Table IV. When these fish are unable to see each other, they are likewise unable to form quiescent schools. This loss of visual inhibition of activity results in increased swimming and consequently greater oxygen consumption.

An analysis of the mean oxygen consumption values of Tables VI, VII, VIII and IX, B, for the blind characins shows a definite increase in oxygen uptake when these animals are tested in the light. This is clearly shown in Text-fig. 2. The means represent 60 tests on the blind animals and the daylight increase in oxygen consumption is consistent throughout. Since locomotor activity is proportional to oxygen consumption, thus evidently swimming speed is greater in the daylight than in the dark. This is consistent with the work of Breder and Gresser (1941b) who demonstrated by means of a gradient trough that the blind fish are slightly but significantly negative to light.

An examination of Text-fig. 2 indicates that in the active state the oxygen consumption values of eyed fish tend to approach that of their blind relatives, both in light and in darkness. This figure also indicates more clearly that light is an extremely important factor in determining the locomotor habits of the animal.

Also shown is the behavior of five immature optically intact fish and five immature blind fish. It will be noted that the two groups show opposite trends in reference to light and darkness. The increased consumption of the eyed fish in the dark is evidently due to a loss of the "group effect."

Text-fig. 2 also indicates that when the blind fish are tested in groups of two their oxygen consumption is lower than when tested individually and the difference between light and darkness is much less. Breder and Rasquin (1943) detected a slight "group effect" in the blind fish. This point is further supported by these lowered oxygen consumption results. Since the oxygen consumption of grouped animals is lowered both in the light and in the dark, it indicates that the "group effect" in the blind animals is due to factors other than light sensitivity. Determining the nature of these factors is beyond the scope of the present paper.



TEXT-FIG. 2. Oxygen consumption charts. The graph covering a five-day period is the data of Table I in which the hourly readings have been weighted to agree with those covering two hours. The large circles connected by a solid line represent the daily mean. The small circles connected by dotted lines represent the daily maximum and minimum reading. The light and dark circles over "Blind" and "Eyed" represent the mean values obtained in light and darkness respectively. In each case they are connected by a solid line. The large numerals indicate the number of fish present in the test chamber. The data are from Tables II to VIII inclusive.

The two optically intact and active fish tested in darkness and shown in Text-fig. 2 show less oxygen consumption than the lone one. This further supports the fact that eyed fish, in the dark, act like their blind relatives. The form of blind fish from Cueva de los Sabinos in which the optic nerve has disappeared and which is indifferent to light, Breder (1944), would be expected, on that basis, to show no change in oxygen consumption correlated with light and darkness. At the time the present experiments were made no specimens of this form were available.

A comparison of the various values obtained do not yield p values, in most cases, necessary to establish an unequivocal statistical separation. However, the biological and statistical validity of these results may be checked in a quite different way, the actual meaning of the p value check in this case indicating that due to the relatively large amount of individual variation 20 tests are not sufficient to show unequivocally separation in that fashion. However, since we already know that eyed characins are largely quiescent in the light due to the principle of optical fixation and when blinded take on the active wandering of the normally blind fish, it is a foregone conclusion that the passive eyed fish would consume more oxygen in the dark than in the light, which they do according to these mean values. Contrariwise the blind and the active eyed fish all agree in reducing their oxygen consump-

Table IX. Comparison of Data.

A.

Light		Dark	
Eyed No. 4 (quiescent)		Eyed No. 4	
Min.	.41	Min.	.54
Aver.	.63	Aver.	.73
Max.	.77	Max.	1.04
Eyed No. 1 (fighting)		Eyed No. 1	
Min.	.98	Min.	.55
Aver.	1.15	Aver.	.92
Max.	1.54	Max.	1.47
		Two Eyed, Nos. 2, 3	
		Min.	1.19 = (0.60) per fish
		Aver.	1.73 = (0.87) per fish
		Max.	2.52 = (1.26) per fish
Five Immature Eyed		Five Immature Eyed	
Min.	.83 = (0.17) per fish	Min.	.97 = (0.19) per fish
Aver.	1.09 = (0.22) per fish	Aver.	1.38 = (0.28) per fish
Max.	1.46 = (0.29) per fish	Max.	1.96 = (0.39) per fish
Blind No. 5		Blind No. 5	
Min.	.90	Min.	.56
Aver.	1.30	Aver.	1.02
Max.	1.67	Max.	1.40
Two Blind Nos. 6, 7		Two Blind Nos. 6, 7	
Min.	1.12 = (0.56) per fish	Min.	1.26 = (0.63) per fish
Aver.	1.95 = (0.98) per fish	Aver.	1.86 = (0.93) per fish
Max.	2.31 = (1.16) per fish	Max.	2.72 = (1.36) per fish
Five Immature Blind		Five Immature Blind	
Min.	.77 = (0.15) per fish	Min.	.70 = (0.14) per fish
Aver.	1.36 = (0.27) per fish	Aver.	1.18 = (0.24) per fish
Max.	2.23 = (0.45) per fish	Max.	1.54 = (0.31) per fish

B.

Comparison of mean values.

Figures in parentheses represent the average per fish of specimens in groups.

EYED

Fish No.	Light	Dark	Difference
4	0.63	0.73	+ 0.10
1	1.15	0.92	— 0.23
Means	*0.89*	*0.83*	*— 0.06*
2, 3	—	1.73 (0.87)	—
five immature	1.09 (0.22)	1.38 (0.28)	+ 0.29 (0.06)

BLIND

Fish No.	Light	Dark	Difference
5	1.30	1.02	— 0.28
6, 7	1.95 (0.98)	1.86 (0.93)	— 0.09 (0.05)
five immature	1.36 (0.27)	1.18 (0.24)	— 0.18 (0.02)

Comparison of like groups.

	Light		Dark		Difference	
Group Size	Eyed	Blind	Eyed	Blind	Eyed	Blind
	1.15		0.92		— 0.23	
1 fish	0.89	1.30	0.83	1.02	— 0.06	— 0.28
	0.63		0.73		+ 0.10	
2 fish	—	0.98	0.87	0.93	—	— 0.05
5 imms.	0.22	0.27	0.28	0.24	+ 0.06	— 0.03

tion in dark. Since there are six separate sets of paired experiments in each case the random chance of reaching this agreement is of inconsequential size. Furthermore, there is an acceptable value for p when the passive eyed fish are compared with either the active eyed and the blind fish and of course with the juveniles, on a size basis.

Hyman (1940) discusses at length the fact that many types of invertebrates show negative phototaxis. A brief survey of invertebrate classes as discussed by Hyman shows that (1) non-green protozoa are negative to light, (2) many medusae indicate perception of light intensity by avoidance of bright sunlight. They appear at the surface in the morning or late afternoon and descend in midday. Clouding of the sky may bring them to the surface at any time. (3) Light accelerates the pulsation rate in normal specimens of *Aurelia* but has no such action after extirpation of the eye spots. (4) Many hydromedusae are indifferent to light, but some have definite responses, accelerating their swimming and exhibiting increased activity in the daylight although avoiding direct sunlight. The blind characins exhibit reactions to light which would seem to be not dissimilar in a broad sense, especially in reference to item 4.

The negative phototaxis exhibited by the blind characins, characterized by an increased oxygen consumption and increased swimming speed, would seem to be a factor leading to cave entry. Since the blind fish swim faster in the light than in the dark, they would automatically gravitate to dark places such as caves and tend to stay there, considering only the mechanical results of such reactions. Since the opposite is true of the eyed fish when in the normal non-fighting condition, they in turn, on the same basis, would tend to stay out of caves.

Obviously in a state of nature the situation would involve many other factors. Thus the coming of night would equalize the light differential within and without caves. This in fact may be an important effect in the entry of eyed fishes into caves with a subsequent inability to find their way out. The presence of predators in the outside waters and their evident absence in caves and numerous other factors increase the complications to be expected in a state of nature but do not override the basic importance of the differentials in locomotor behavior between the seeing and the blind fish as here demonstrated.

SUMMARY.

1. The eyed river fish exhibit two very distinct phases of behavior, a turbulent fighting state and a quiescent state.

2. In light the blind cave fish, due to a constant wandering activity, consume more oxygen than eyed river fish, when the latter are in a quiescent schooling state.

3. In total darkness, eyed fish in the resting state increase their oxygen consumption to a point where it approaches that of the blind fish. The above is interpreted to indicate that eyed fish assume the wandering behavior of the blind animals when in the dark.

4. Eyed fish in the highly active state consume less oxygen in the dark than in the light. The decreased oxygen consumption in the dark approaches the values obtained for the blind fish.

5. The contrasting locomotor behavior of the eyed and blind fish, as evidenced by their differential locomotor activity, is clearly based on vision and its lack.

6. The blind fish consume more oxygen in the light than in the dark. This indicates a greater swimming speed in the light which would tend to cause these fish to spend more time in the dark. This would seem to be a factor in cave entry and cave retention. The eyed fish, when in the non-fighting condition, show opposite effects with an opposite relationship to cave entry.

7. Two blind fish, in a given volume of water, consume less oxygen than when tested individually in the same volume of water thus evidencing the "group effect" common to other forms and indicating the extent of these effects on each other.

8. It is impossible to test two optically intact fish in the same chamber, since they destroy each other, evidently because of too great a constrained association.

BIBLIOGRAPHY.

ADKINS, M.

1930. A method for determining basal metabolism of fishes. *Proc. Soc. Exp. Biol. Med.*, vol. 28, pp. 259-263.

ALLEE, W. C., AND R. OESTING

1934. A critical examination of Winkler's method for determining dissolved oxygen in respiration studies with aquatic animals. *Physiol. Zööl.*, vol. 7, pp. 509-541.

AMERICAN PUBLIC HEALTH ASSOCIATION

1943. Standard methods for the examination of water and sewage. Eighth edition. New York, xiv + 309 pp.

BREDER, C. M., JR.

1942. Descriptive ecology of La Cueva Chica, with especial reference to the blind fish, *Anoptichthys*. *Zoologica*, New York, vol. 27, pp. 7-15.

1943. A note on erratic viciousness in *Astyanax mexicanus* (Phillipi). *Copeia*, no. 2, pp. 82-84.

1944. Ocular anatomy and light sensitivity studies on the blind fish from Cueva de los Sabinos. *Zoologica*, New York, vol. 29, pp. 131-143.

BREDER, C. M., JR., AND E. B. GRESSER
1941a. Correlations between structural eye defects and behavior in the Mexican blind characin. *Zoologica*, New York, vol. 26, pp. 123-131.
1941b. Further studies in the light sensitivity and behavior of the Mexican blind characins. *Ibid.*, vol. 26, pp. 289-296.

BREDER, C. M., JR., AND R. F. NIGRELLI
1938. The significance of differential locomotor activity as an index to the mass physiology of fishes. *Zoologica*, New York, vol. 23, pp. 1-29.

BREDER, C. M., JR., AND P. RASQUIN
1943. Chemical sensory reactions in the Mexican blind characins. *Zoologica*, New York, vol. 28, pp. 169-200.

ESCOBAR, R. A., R. P. MINAHAN, AND R. J. SHAW
1936. Motility factors in mass physiology: locomotor activity of fishes under conditions of isolation, homotypic grouping, and heterotypic grouping. *Physiol. Zööl.*, vol. 9, pp. 66-78.

ETKIN, W., R. W. ROOT, AND B. P. MOFSHIN
1940. The effect of thyroid feeding on oxygen consumption of the goldfish. *Physiol. Zööl.*, vol. 13, pp. 415-429.

HUBBS, C. L., AND W. T. INNES
1936. The first known blind fish from the family Characidae: a new genus from Mexico. *Occas. Papers Mus. Zool. Univ. Michigan*, no. 342, pp. 1-7.

HYMAN, L. H.
1940. The invertebrates: Protozoa through Ctenophora. New York, xi + 726 pp.

KEYS, A. B.
1930. The measurement of the respiratory exchange of aquatic animals. *Biol. Bull.*, vol. 59, pp. 187-198.

SCHLAIFER, A.
1938. Studies in mass physiology: effect of numbers upon the oxygen consumption and locomotor activity of *Carassius auratus*. *Physiol. Zööl.*, vol. 11, pp. 408-424.
1939. An analysis of the effect of numbers upon the oxygen consumption of *Carassius auratus*. *Ibid.*, vol. 12, pp. 381-392.

SCHUETT, J. F.
1933. Studies in mass physiology: the effect of numbers upon the oxygen consumption of fishes. *Ecology*, vol. 14, pp. 106-122.
1934. Studies in mass physiology: the activity of goldfish under different conditions of aggregation. *Ibid.*, vol. 15, pp. 258-262.

SHAW, R. J., R. A. ESCOBAR, AND F. M. BALDWIN
1938. The influence of temperature and illumination on the locomotor activity of *Carassius auratus*. *Ecology*, vol. 19, pp. 343-346.

WELLS, N. A.
1935. The influence of temperature upon the respiratory metabolism of the Pacific killifish, *Fundulus parvipinnis*. *Physiol. Zööl.*, vol. 8, pp. 196-227.